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Mediatech is a leading manufacturer of cell culture media and reagents. Our products are sold direct and through distributors under the trade names cellgro ® and Insectagro ™. Since 1984, we have provided the scientific research and biopharmaceutical production communities with superior products at the lowest prices.

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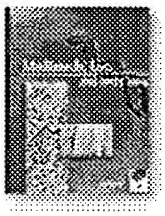

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another Coco Design

Cryopreservation

Cryopreservation is the technique of freezing cells and tissues at very low temperatures at which the biological material remains genetically stable and metabolically inert, while minimizing ice crystal formation. In general, when a tissue is subjected to low temperatures ice crystals will eventually form which will disrupt the cell membrane leading to the death of the cell. The goal of cryopreservation is to replace some of the water with other compounds that will not form large crystals when frozen. The most commonly used replacements are DMSO (dimethylsulfoxide) and glycerol. These are mixed into a solution with media or serum and cells are suspended in it and placed in a liquid nitrogen freezer. As the media begins to freeze the salt concentration outside the cells will become greater than that in the cells and water will leave the cells to be replaced by the DMSO or glycerol.

Cryopreservation media consists of a base medium, protein source, and a cryopreservative. The cryopreservative both protects the cells from mechanical and physical stress and reduces the water content within the cells, thus minimizing the formation of cell-lysing ice crystals. The protein source, usually fetal bovine serum (FBS) also protects the cells from the stress associated with the freeze-thaw process. Cells are frozen slowly, at 1°C/minute using programmable coolers or by techniques outlined below.

Generally, the optimum cell density to freeze per 1ml of cell suspension depends on the type of cell. Mammalian cells are usually frozen between $1x10^6$ cells/ml to $1x10^7$ cells/ml. The cryopreservation media differs slightly for adherent and suspension cell types. The following cryo media is generally used:

ADHERENT CELL TYPES	SUSPENSION CELL TYPES
90% Fresh Medium (including protein)	45% Fresh medium (including protein)
10% DMSO (or other cryopreservative)	45% Spent (used) medium
	10% DMSO (or other cryopreservative)

For more information regarding cyopreservation, or other technical information, Please call Mediatech's Technical Service department

Call: **800-cellgro** (235-5476)

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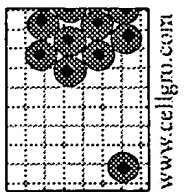

Cryopreservation Protocol

Suspension Cultures

1. Expand culture to allow for adequate cell density for the desired volume to freeze. Optimum conditions include cells currently in the log phase of the growth cycle (approximately 2-4 days after subculturing).
2. Combine the cell suspensions (multiple flasks) into a single sterile vessel (single culture flask, for example). From this single suspension, count the cells to determine cell viability and total cell concentration.
3. Centrifuge cells at approximately 200 to 400 x g for 10 minutes, allowing the cells to form a pellet. During centrifugation, determine the amount of freezing media to prepare.

   For example, if using 1ml cryovials, divide the total cell concentration by the desired cell density. This number indicates the number of possible 1ml cryovials which may be frozen at the desired cell density; this is also the number of mls of cryopreservation media to prepare.

   Example:

   A $4x10^7$ cell suspension will yeild a total of 10 1ml alliquots at $4x10^6$ cells per alliquot.

   Prepare 10ml of freezing media to easily suspend the pellet at the correct cell density.

   (cell suspension) / (desired freezing density) = # of possible 1ml alliquots at desired density (also the amount of freezing media to prepare)
4. When centrifugation is complete, aseptically transfer the supernatant (spent media) to a sterile container and set aside. Some of this medium will be used to prepare the cryopreservation medium.
5. Prepare the necessary volume of cryopreservation media (determined above) using 45% fresh complete medium (including protein source, usually FBS), 45% spent media (supernatant), and 10% DMSO or other cryopreservative.
6. Resuspend cell pellet(s) using the cryopreservation media, triturating to ensure a single-cell suspension/as few cell clumps as possible.
7. Dispense into the desired number of vials for cryopreservation.
8. Immediately transfer the vials to a freezer with a minimum temperature of -20°C for one hour.
9. Transfer the vials to a -80°C freezer for 24 hours. Alternatively, a dry ice/methanol slurry (-80°C) using a styrofoam or other insulated box with a cover or lid may be used if a -80°C freezer is not available.
10. After 24 hours at -80°C, cells may be transferred to liquid nitrogen storage (-196°C).

Adherent Cultures

1. Expand culture to allow for adequate cell density for the desired volume to freeze. Optimum conditions include cells currently in the log phase of the growth cycle (approximately 2-4 days after subculturing).
2. Gently detach cells from the substrate using dissociation solutions.
3. Resuspend the detached cells in a complete growth medium.
4. Perform a cell count to determine the number of viable cells and the total cell concentration.
5. Centrifuge cells at approximately 200 to 400 x g for 10 minutes, allowing the cells to form a pellet. During centrifugation, determine the amount of freezing media to prepare.

   For example, if using 1ml cryovials, divide the total cell concentration by the desired cell density. This number indicates the number of possible 1ml cryovials which may be frozen at the desired cell density; this is also the number of mls of cryopreservation media to prepare.

   Example:

   A $4x10^7$ cell suspension will yeild a total of 10 1ml alliquots at $4x10^6$ cells per alliquot.

   Prepare 10ml of freezing media to easily suspend the pellet at the correct cell density.

   (cell suspension) / (desired freezing density) = # of possible 1ml alliquots at desired density (also the amount of freezing media to prepare)
6. Prepare the necessary volume of cryopreservation media (determined above) using 90% fresh complete medium (including protein source, usually FBS) and 10% DMSO or other cryopreservative.
7. Resuspend cell pellet(s) using the cryopreservation media, triturating to ensure a single-cell suspension/as few cell clumps as possible.
8. Dispense into the desired number of vials for cryopreservation.
9. Immediately transfer the vials to a freezer with a minimum temperature of -20°C for one hour.
10. Transfer the vials to a -80°C freezer for 24 hours. Alternatively, a dry ice/methanol slurry (-80°C) using a styrofoam or other insulated box with a cover or lid may be used if a -80°C freezer is not available.
11. After 24 hours at -80°C, cells may be transferred to liquid nitrogen storage (-196°C).